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Inhibition of Ras Signaling in Neurofibromitosis.

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14. ABSTRACT

The purpose of the proposed research is to screen for small molecules that interfere with the signaling of Ras in Neurofibromitosis. During this research period, an 'open conformation' of Ras that is non-signaling has been generated. The current hypothesis is that this new conformation is adopted by Ras in the GTP-bound form before it adopts its active effector-binding conformation and therefore is a target for inhibition by small molecules that would attenuate the signaling of hyper-activated Ras in Neurofibromitosis and in other human cancers. Small molecules are being screened for their ability to inhibit or decrease the in vitro binding of Ras to downstream effectors including Raf.

15. SUBJECT TERMS

Ras, neurofibromatosis, cycling, activation, GTP-binding

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Introduction and Significance

There is now compelling evidence that aberrant Ras and Ras-like GTPases signaling contributes to many human diseases especially cancer and X-linked neurodegenerative diseases. In these disorders, Ras is constitutively active or GTP-bound either because of a mutation that slows its ability to hydrolyze GTP or because an upstream regulator is constitutively activated (e.g. a membrane-bound receptor such as EGFR). Despite the accumulated knowledge and decades of research, few available drugs directly target Ras or Ras-like GTPases. A need for such a drug(s) is urgent for example to fight Neurofibromitosis where K-Ras is predominantly activated. We argue that the lack of anti-Ras drugs lays in the lack of understanding of the path for Ras cycling between the GTP- and GDP-bound forms. We hypothesize that describing the path for GTP hydrolysis and the path for nucleotide exchange will help design new molecules that stabilize Ras in a non-signaling conformation.

Key Research Accomplishments:

1- To investigate the cycling of Ras between the active and inactive forms (Aim 1). We have continued to study the cycling of Ras between the GTP- and GDP-bound forms by manipulating the flexibility of the switch regions. Recent published work by other investigators suggests that our data especially the ones related to the 'open conformation' of Ras are not an experimental artifact but are transient conformations of Ras along its path between the active and inactive forms. For example, recent molecular dynamics results by the McCammon group (see Lukman *et al*., 2010; Grant *et al*., 2009; Gorfe *et al*., 2008) confirm our approach. Most importantly, the structure of GTP-bound RasT35S in the 'inactive state 1' recently reported by Shima *et al*. (2010) is remarkably similar to the open conformation we found. In the 'open conformation', the switch 1 and 2 domains adopt a conformation that are reminiscent of nucleotide free Ras despite the presence of GDP or GTP. This opening of the switch regions results in the appearance of new pockets on Ras that can be used to dock small molecule inhibitors. Overall, these findings validate our hypothesis and support our approach of screening the open conformation of Ras to find new Ras inhibitors that could eventually be developed into anti-cancer drugs. We are currently running a high throughput screen (HTS) searching a library for small molecules that bind to the open conformation of Ras. Since last report in 2009, we have published our work on the dominant negative form of Ras, RasS17N, (Nassar *et al*, 2010). We have submitted our molecular dynamics results obtained in collaboration with Dr. Jin Wang (Chemistry, Stony Brook) regarding the reaction of GTP-hydrolysis catalyzed by Ras as investigated using a new-targeted molecular dynamics algorithm for publication (Lu *et al.,* 2011 in review)

2- In addition, we are collaborating with Dr. Francesco Peri (Italy), who has developed a series of Ras inhibitors, in order to improve on the binding and specificity of his compounds.

3- Other studies. In collaboration with Dr. Nick Carpino (Microbiology, Stony Brook University), we are studying the structure-function relationship of the Sts proteins, which act downstream of the T cell receptor (TCR) complex. We previously showed that Sts-1 is the prototype of a new family of protein tyrosine phosphatases (PTPs) (Mikhailik *et al.,* 2007). We have published the crystal structure of the phosphatase domain of Sts-1, Sts-1_{PGM}, in complex with a sulfate moiety (Jankoncic *et al.,* 2010). The postdoctoral fellow in charge of the Ras project contributed to demonstrating that Sts-2 regulates the level of tyrosine phosphorylation on targets within T cells, among them the critical T cell tyrosine kinase Zap-70 (Boris *et al*., 2011).

In paralle, we demonstrated that Sts-1 and Sts-2 are instrumental in down-modulating proteins that are dually modified by both protein tyrosine phosphorylation and ubiquitination (Carpino *et al*., 2009). Specifically, both naïve and activated T cells derived from genetically engineered mice that lack the Sts proteins display strikingly elevated levels of tyrosine phosphorylated, ubiquitinated proteins following TCR stimulation. The accumulation of the dually modified proteins is transient, and in activated T cells but not naïve T cells is significantly enhanced by co-receptor engagement. These observations hint at a novel regulatory mechanism downstream of the T cell receptor

Publications (total of 4 for this progress period).

1. Nick Carpino, Yunting Chen, Nicolas Nassar and Hye-Won Oh "The Sts proteins target tyrosine phosphorylated, ubiquitinated proteins within TCR signaling pathways" *Mol. Immunol* 46, 3224-3231 (2009).
2. Jakoncic J, Sondgeroth B, Carpino N, Nassar N. "The 1.35 Å resolution structure of the phosphatase domain of the suppressor of T-cell receptor signaling protein in complex with sulfate" *Acta Crystallogr Sect F Struct Biol Cryst Commun*. 2010 Jun 1;66(Pt 6):643-7.
3. San Luis, Boris, Ben Sondgeroth, Nicolas Nassar, and Nick Carpino "STS-2 IS A PHOSPHATASE THAT NEGATIVELY REGULATES ZETA- ASSOCIATED PROTEIN (ZAP)-70 AND T CELL RECEPTOR (TCR) SIGNALING PATHWAYS" in revision *J. Biol. Chem*.
4. Qiang Lu, Nicolas Nassar and Jin Wang "An investigation of Ras catalyzed GTP hydrolysis using classical and quantum targeted molecular dynamics" submitted to *JACS*.